



Donor 4991

Genetic Testing Summary

Fairfax Cryobank recommends reviewing this genetic testing summary with your healthcare provider to determine suitability.

Last Updated: 09/23/22

Donor Reported Ancestry: Polish, French, Irish

Jewish Ancestry: No

Genetic Test*	Result	Comments/Donor's Residual Risk**
Chromosome analysis (karyotype)	Normal male karyotype	No evidence of clinically significant chromosome abnormalities
Hemoglobin evaluation	Normal hemoglobin fractionation and MCV/MCH results	Reduced risk to be a carrier for sickle cell anemia, beta thalassemia, alpha thalassemia trait (aa/-- and a-/a-) and other hemoglobinopathies
Cystic Fibrosis (CF) carrier screening	Negative by genotyping of 149 mutations in the CFTR gene	1/496
Spinal Muscular Atrophy (SMA) carrier screening	Negative for deletions of exon 7 in the SMN1 gene	1/632
Tay Sachs enzyme analysis	Non-carrier by Hexosaminidase A activity	
Standard testing attached- 22 diseases by genotyping	Negative for mutations tested	
Special testing		
Phenylalanine Hydroxylase Deficiency	Carrier: by gene sequencing of the PAH gene	Carrier testing is recommended for those using this donor
Carnitine Palmitoyltransferase II Deficiency	Negative by gene sequencing in the CPT2 gene	1/670
Neuronal Ceroid-Lipofuscinosis: CLN3 Related	Negative by gene sequencing in the CLN3 gene	1/4300
Lamellar Ichthyosis Type 1	Negative by gene sequencing in the TGM1 gene	1/1100
Glycogen Storage Disease Type 3	Negative by gene sequencing in the GAA gene	1/520
Usher Syndrome 2A	Negative by gene sequencing in the USH2A gene	1/314

Myoneurogastrointestinal Encephalopathy	Negative by gene sequencing in the TYMO gene	1/1611
Smith-Lemli-Opitz Syndrome	Negative by gene sequencing in the DHCR7 gene	1/248
Primary Hyperoxaluria Type 1	Negative by gene sequencing in the AGXT gene	1/684
Primary Ciliary Dyskinesia	Negative by gene sequencing in the DNA12 gene	1/9981
Biotinidase Deficiency	Negative by gene sequencing in the BTB gene	1/56
Congenital Adrenal Hypoplasia due to 21-hydroxylase deficiency	Negative by gene sequencing and copy number in the CYP21A2 gene	1/1300- Classic 1/200-Non-Classic
Genes: SLC12A3, ALDOB	Negative by gene sequencing	See attached results for residual risks

*No single test can screen for all genetic disorders. A negative screening result significantly reduces, but cannot eliminate, the risk for these conditions in a pregnancy.

**Donor residual risk is the chance the donor is still a carrier after testing negative.

Ordering Practice:

Practice Code: [REDACTED]

Fairfax Cryobank - [REDACTED]

Physician: [REDACTED]

Report Generated: 2016-05-03

4991 [REDACTED]

DOB: [REDACTED]

Gender: Male

Ethnicity: European

Procedure ID: 51747

Kit Barcode: [REDACTED]

Specimen: Blood, #54553

Specimen Collection: 2016-04-26

Specimen Received: 2016-04-27

Specimen Analyzed: 2016-05-03

Partner Not Tested

TEST INFORMATION

Test: CarrierMap^{GEN} (Genotyping)

Panel: Fairfax Cryobank Panel V2

Diseases Tested: 22

Genes Tested: 22

Mutations Tested: 450

SUMMARY OF RESULTS: NO MUTATIONS IDENTIFIED

4991 [REDACTED] was not identified to carry any of the mutation(s) tested.

No pathogenic mutations were identified in the genes tested, reducing but not eliminating the chance to be a carrier for the associated genetic diseases. CarrierMap assesses carrier status for genetic disease via molecular methods including targeted mutation analysis and/ or next-generation sequencing; other methodologies such as CBC and hemoglobin electrophoresis for hemoglobinopathies and enzyme analysis for Tay-Sachs disease may further refine risks for these conditions. Results should be interpreted in the context of clinical findings, family history, and/or other testing. A list of all the diseases and mutations screened for is included at the end of the report. This test does not screen for every possible genetic disease.

For additional disease information, please visit recombine.com/diseases. To speak with a Genetic Counselor, call [855.OUR.GENES](tel:855.OUR.GENES).

Assay performed by

Reprogenetics

CLIA ID: 31D1054821

3 Regent Street, Livingston, NJ 07039

Lab Technician: Bo Chu

Recombine CLIA # 31D2100763

Reviewed by Pere Colls, PhD, HCLD, Lab Director

ADDITIONAL RESULTS: NO INCREASED REPRODUCTIVE RISK

The following results are not associated with an increased reproductive risk.

Disease (Gene)	4991 [REDACTED]	Partner Not Tested
Spinal Muscular Atrophy: SMN1 Linked (SMN1) *	SMN1 Copy Number: 2 or more copies Method: Genotyping & dPCR	

* SMA Risk Information for Individuals with No Family History of SMA

	Detection Rate	Pre-Test Carrier Risk	Post-Test Carrier Risk (2 SMN1 copies)	Post-Test Carrier Risk (3 SMN1 copies)
European	95%	1/35	1/632	1/3,500
Ashkenazi Jewish	90%	1/41	1/350	1/4,000
Asian	93%	1/53	1/628	1/5,000
African American	71%	1/66	1/121	1/3,000
Hispanic	91%	1/117	1/1,061	1/11,000

For other unspecified ethnicities, post-test carrier risk is assumed to be <1%. For individuals with multiple ethnicities, it is recommended to use the most conservative risk estimate.

Methods and Limitations

Genotyping: Genotyping is performed using the Illumina Infinium Custom HD Genotyping assay to identify mutations in the genes tested. The assay is not validated for homozygous mutations, and it is possible that individuals affected with disease may not be accurately genotyped.

Spinal Muscular Atrophy: Carrier status for SMA is assessed via copy number analysis by dPCR and via genotyping. Some individuals with a normal number of SMN1 copies (2 copies) may carry both copies of the gene on the same allele/chromosome; this analysis is not able to detect these individuals. Thus, a normal SMN1 result significantly reduces but does not eliminate the risk of being a carrier. Additionally, SMA may be caused by non-deletion mutations in the SMN1 gene; CarrierMap tests for some, but not all, of these mutations. Some SMA cases arise as the result of de novo mutation events which will not be detected by carrier testing.

Limitations: In some cases, genetic variations other than that which is being assayed may interfere with mutation detection, resulting in false-negative or false-positive results. Additional sources of error include, but are not limited to: sample contamination, sample mix-up, bone marrow transplantation, blood transfusions, and technical errors. The test does not test for all forms of genetic disease, birth defects, and intellectual disability. All results should be interpreted in the context of family history; additional evaluation may be indicated based on a history of these conditions. Additional testing may be necessary to determine mutation phase in individuals identified to carry more than one mutation in the same gene. All mutations included within the genes assayed may not be detected, and additional testing may be appropriate for some individuals.

This test was developed and its performance determined by Recombine, Inc., and it has not been cleared or approved by the U.S. Food and Drug Administration (FDA). The FDA has determined that such clearance or approval is not necessary.

Diseases & Mutations Assayed

Alpha Thalassemia : Mutations (9): ♂ Genotyping | SEA deletion, c.207C>A (p.N69K), c.223G>C (p.D75G), c.21T>C (p.M11T), c.207C>G (p.N69K), c.340_351delCTCCCGCCGAG (p.L114_ E117del), c.377T>C (p.L126P), c.427T>C (p.X143Qex32), c.*+94A>G

Beta Thalassemia : Mutations (82): ♂ Genotyping | c.124_127delTTCT (p.F42Lfs), c.17_18delCT, c.20delA (p.E7Gfs), c.217insA (p.S73Kfs), c.223+702_444+342del620insAAGTAGA, c.230delC, c.25_26delAA, c.315+1G>A, c.315+2T>C, c.316-197C>T, c.316-146T>G, c.315+745C>G, c.316-1G>A, c.316-1G>C, c.316-2A>G, c.316-3C>A, c.316-3C>G, c.4delG (p.V2Cfs), c.51delC (p.K18Rfs), c.93-21G>A, c.92+1G>A, c.92+5G>A, c.92+5G>C, c.92+5G>T, c.92+6T>C, c.93-1G>A, c.93-1G>T, c.-50A>C, c.-78a>g, c.-79a>g, c.-81a>g, c.52A>T (p.K18X), c.-137C>g, c.-138C>t, c.-151C>t, c.118C>T (p.Q40X), c.169G>C (p.G57R), c.295G>A (p.V99M), c.34G>A (p.V12I), c.415G>C (p.A139P), c.47G>A (p.W16X), c.48G>A (p.W16X), c.-80P>a, c.2T>C (p.M11T), c.75T>A (p.G25G), c.444+111A>G, c.-29g>a, c.68_74delAAGTTGG, c.92G>C (p.R31T), c.92+1G>T, c.93-15T>G, c.93-1G>C, c.112delT, c.113G>A (p.W38X), c.114G>A (p.W38X), c.126delC, c.444+113A>G, c.250delG, c.225delC, c.383_385delAGG (p.Q128_A129delQAinsP), c.321_322insG (p.N109fs), c.316-1G>T, c.316-2A>C, c.287_288insA (p.L97fs), c.271G>T (p.E91X), c.203_204delTTG (p.V68Afs), c.154delC (p.P52fs), c.135delC (p.F46fs), c.92+2T>A, c.92+2T>C, c.90C>T (p.G30G), c.84_85insC (p.L29fs), c.59A>G (p.N20S), c.46delT (p.W16Gfs), c.45_46insG (p.L16fs), c.36delT (p.T13fs), c.2T>G (p.M1R), c.1A>G (p.M1V), c.-137C>t, c.-136C>g, c.-142C>t, c.-140C>t

Bloom Syndrome : Mutations (24): ♂ Genotyping | c.2207_2212delATCTGAGinsTAGATTC (p.Y736Lfs), c.2407insT, c.557_559delCAA (p.S186X), c.1284G>A (p.W428X), c.1701G>A (p.W567X), c.1933C>T (p.Q645X), c.2528C>T (p.T843I), c.2695C>T (p.R899X), c.3107G>T (p.C1036F), c.2923delC (p.Q975K), c.3558+1G>T, c.3875-2A>G, c.2074+2T>A, c.2343_2344dupGA (p.T81EfsX), c.380delC (p.127Tfs), c.3564delC (p.1188Dfs), c.4008delG (p.1336Rfs), c.947C>G (p.S316X), c.2193+1_2193+9del9, c.1642C>T (p.G548X), c.3143delA (p.1048NfsX), c.356_357delTA (p.C120Hfs), c.4076+1delG, c.3281C>A (p.S1094X)

Canavan Disease : Mutations (8): ♂ Genotyping | c.433-2A>G, c.854A>C (p.E285A), c.693C>A (p.Y231X), c.914C>A (p.A305E), c.71A>G (p.E24G), c.654C>A (p.C218X), c.2T>C (p.M11T), c.79G>A (p.G27R)

Cystic Fibrosis : Mutations (149): ♂ Genotyping | c.1029delC, 1153_1154insAT, c.1477delCA, c.1519_1521delATC (p.S507delI), c.1521_1523delCTT (p.S508delF), c.1545_1546delATA (p.Y515fs), c.1585-1G>A, c.164+12T>C, c.1680-886A>G, c.1680-1G>A, c.1766+1G>A, c.1766+1G>T, c.1766+5G>T, c.1818del84, c.1911delG, c.1923delCTCAAAATCinsA, c.1973delGAAATTCATCTCinsAGAAA, c.2052delA (p.K684fs), c.2052insA (p.Q685fs), c.2051_2052delAAinsG (p.K684SfsX38), c.2174insA, c.261delTT, c.2657+5G>A, c.273+1G>A, c.273+3A>C, c.274-1G>A, c.2988+1G>A, c.3039delC, c.3140-26A>G, c.325delATinsG, c.3527delC, c.3535delACCA, c.3691delT, c.3717+1291C>T, c.3744delA, c.3773_3774insT (p.L1258fs), c.442delA, c.489+1G>T, c.531delT, c.579+1G>T, c.579+5G>A (IVS4+5G>A), c.803delA (p.N268fs), c.805_806delAT (p.I269fs), c.933_935delCTT (p.S11delF), c.946delT, c.1645A>C (p.S549R), c.2128A>T (p.K710X), c.1000C>T (p.R334W), c.1013C>T (p.T338I), c.1364C>A (p.A455E), c.1477C>T (p.Q493X), c.1572C>A (p.C524X), c.1654C>T (p.Q552X), c.1657C>T (p.R553X), c.1721C>A (p.P574H), c.2125C>T (p.R709X), c.223C>T (p.R75X), c.2668C>T (p.Q890X), c.3196C>T (p.R1066C), c.3276C>G (p.Y1092X), c.3472C>T (p.R1158X), c.3484C>T (p.R1162X), c.349C>T (p.R117C), c.3587C>G (p.S1196X), c.3712C>T (p.Q1238X), c.3764C>A (p.S1255X), c.3909C>G (p.N1303K), c.1040G>A (p.R347H), c.1040G>C (p.R347P), c.1438G>T (p.G480C), c.1558G>T (p.V520F), c.1624G>T (p.G542X), c.1646G>A (p.S549N), c.1646G>T (p.S549I), c.1652G>A (p.G551D), c.1675G>A (p.A559T), c.1679G>C (p.R560T), c.178G>T (p.E60X), c.1865G>A (p.G622D), c.254G>A (p.G85E), c.271G>A (p.G91R), c.274G>T (p.E92X), c.3209G>A (p.R1070Q), c.3266G>A (p.W1089X), c.3454G>C (p.D1152H), c.3500G>A (p.R117H), c.3611G>A (p.W1204X), c.3752G>A (p.S1251N), c.3846G>A (p.W1282X), c.3848G>T (p.R1283M), c.532G>A (p.G178R), c.988G>T (p.G330X), c.1090T>C (p.S364P), c.3302T>A (p.M1101K), c.617T>G (p.L206W), c.14C>T (p.P5L), c.19G>T (p.E7X), c.171G>A (p.W57X), c.313delA (p.I105fs), c.328G>C (p.D110H), c.580-1G>T, c.1055G>A (p.R352Q), c.1075C>A (p.Q359K), c.1079C>A (p.T360K), c.1647T>G (p.S549R), c.1976delA (p.N659fs), c.2290C>T (p.R764X), c.2737_2738insG (p.Y913X), c.3067_3072delATAGTG (p.I1023_V1024delT), c.3536_3539delCCAA (p.T1179fs), c.3659delC (p.T1220fs), c.54-5940_273+10250del21080bp (p.S18fs), c.4056G>C (p.Q1352H), c.4364C>G (p.S1455X), c.4003C>T (p.L1335F), c.2538G>A (p.W846X), c.200C>T (p.P67L), c.4426C>T (p.Q1476X), c.1116+1G>A, c.1986_1989delAACT (p.T663R), c.2089_2090insA (p.R697Kfs), c.2215delG (p.V739Y), c.263T>G (p.L196X), c.3022delG (p.V1008S), c.3908dupA (p.N1303Kfs), c.658C>T (p.Q220X), c.868C>T (p.Q290X), c.1526delG (p.G509fs), c.2908+1085-3367+260del7201, c.11C>A (p.S4X), c.3700A>G (p.I1234V), c.416A>T (p.H139L), c.366T>A (p.Y122X), c.3767_3768insC (p.A1256fs), c.613C>T (p.P205S), c.293A>G (p.Q98R), c.3731G>A (p.G1244E), c.535C>A (p.Q179K), c.3368-2A>G, c.455T>G (p.M152R), c.1610_1611delAC (p.D537fs), c.3254A>G (p.H1085R), c.496A>G (p.K166E), c.1408_1417delGTGATTATGG (p.V470fs), c.1585-8G>A, c.2909G>A (p.G970D), c.653T>A (p.L218X), c.1175T>G (p.V392G), c.3139_3139+1delGG

Familial Dysautonomia : Mutations (4): ♂ Genotyping | c.2204+6T>C, c.2741C>T (p.P914L), c.2087G>C (p.R696P), c.2128C>T (p.Q710X)

Familial Hyperinsulinism: Type 1: ABCC8 Related : Mutations (10): ♂ Genotyping | c.3989-9G>A, c.4159_4161delITTC (p.I387delF), c.4258C>T (p.R1420C), c.4477C>T (p.R1493W), c.2147G>T (p.G716V), c.4055G>C (p.R1352P), c.560T>A (p.V187D), c.4516G>A (p.E1506K), c.2506C>T (p.Q836X), c.579+2T>A

Fanconi Anemia: Type C : Mutations (8): ♂ Genotyping | c.456+4A>T, c.67delG, c.37C>T (p.Q13X), c.553C>T (p.R185X), c.1661T>C (p.L554P), c.1642C>T (p.R548X), c.66G>A (p.W22X), c.65G>A (p.W22X)

Gaucher Disease : Mutations (6): ♂ Genotyping | c.84_85insG, c.1226A>G (p.N409S), c.1343A>T

(p.D448V), c.1504C>T (p.R502C), c.1297G>T (p.V433L), c.1604G>A (p.R535H)

Glycogen Storage Disease: Type IA : Mutations (13): ♂ Genotyping | c.376_377insTA, c.79delC, c.979_981delITTC (p.327delF), c.1039C>T (p.Q347X), c.247C>T (p.R83C), c.724C>T (p.Q242X), c.248G>A (p.R83H), c.562G>C (p.G188R), c.648G>T, c.809G>T (p.G270V), c.113A>T (p.D38V), c.975delG (p.L326fs), c.724delC

Joubert Syndrome : Mutations (2): ♂ Genotyping | c.218G>T (p.R73L), c.218G>A (p.R73H)

Maple Syrup Urine Disease: Type 1B : Mutations (6): ♂ Genotyping | c.1114G>T (p.E372X), c.548G>C (p.R183P), c.832G>A (p.G278S), c.970C>T (p.R324X), c.487G>T (p.E163X), c.853C>T (p.R285X)

Maple Syrup Urine Disease: Type 3 : Mutations (8): ♂ Genotyping | c.104_105insA, c.685G>T (p.G229C), c.214A>G (p.K72E), c.1081A>G (p.M361V), c.1123G>A (p.E375K), c.1178T>C (p.I393T), c.1463C>T (p.P488L), c.1483A>G (p.R495G)

Mucopolidiosis: Type IV : Mutations (5): ♂ Genotyping | c.-1015_788del6433, c.406-2A>G, c.1084G>T (p.D362Y), c.304C>T (p.R102X), c.244delC (p.L82fsX)

Nemaline Myopathy: NEB Related : Mutations (1): ♂ Genotyping | c.7434_7536del2502bp

Niemann-Pick Disease: Type A : Mutations (6): ♂ Genotyping | c.996delC, c.1493G>T (p.R498L), c.911T>C (p.L104P), c.1267C>T (p.H423Y), c.1734G>C (p.K578N), c.1493G>A (p.R498H)

Sickle-Cell Anemia : Mutations (1): ♂ Genotyping | c.20A>T (p.E7V)

Spinal Muscular Atrophy: SMN1 Linked : Mutations (19): ♂ Genotyping | DEL EXON 7, c.22_23insA, c.43C>T (p.Q15X), c.91_92insT, c.305G>A (p.W102X), c.400G>A (p.E134K), c.439_443delGAAGT, c.558delA, c.585_586insT, c.683T>A (p.L228X), c.734C>T (p.P245I), c.768_778dupTGCTGATGCTT, c.815A>G (p.Y272C), c.821C>T (p.T274I), c.823G>A (p.G275S), c.834+2T>G, c.835-18_835-12delCCTTTAT, c.835G>T, c.836G>T dPCR | DELE EXON 7

Tay-Sachs Disease : Mutations (76): ♂ Genotyping | c.1073+1G>A, c.1277_1278insATC, c.1421+1G>C, c.805+1G>A, c.532C>T (p.R178C), c.533G>A (p.R178H), c.805G>A (p.G269S), c.1510C>T (p.R504C), c.1496G>A (p.R499H), c.509G>A (p.R170Q), c.1003A>T (p.I335F), c.910_912delITTC (p.305delF), c.749G>A (p.G250D), c.632T>C (p.F211S), c.629C>T (p.S210F), c.613delC, c.611A>G (p.H204R), c.598G>A (p.V200M), c.590A>C (p.K197T), c.571-1G>T, c.540C>G (p.Y180X), c.538T>C (p.Y180H), c.533G>T (p.R178L), c.508C>T (p.R170W), c.409C>T (p.R137X), c.380T>G (p.L127R), c.346+1G>C, c.116T>G (p.L39R), c.78G>A (p.W26X), c.1A>G (p.M1V), c.1495C>T (p.R499C), c.459+5G>A (IVS4+5G>A), c.1422-2A>G, c.535C>T (p.H179Y), c.1141delG (p.V381fs), c.796T>G (p.W266G), c.155C>A (p.S52X), c.426delT (p.F142fs), c.413-2A>G, c.570+3A>G, c.536A>G (p.H179R), c.1146+1G>A, c.736G>A (p.A246T), c.1302C>G (p.F434L), c.778C>T (p.P260S), c.1008G>T (p.Q336H), c.1385A>T (p.E462V), c.964G>A (p.D322N), c.340G>A (p.E114K), c.1432G>A (p.G478R), c.1178G>C (p.R393P), c.805+1G>C, c.1426A>T (p.R476X), c.623A>T (p.D208V), c.1537C>T (p.Q513X), c.1511G>T (p.R504L), c.1307_1308delATA (p.I436fs), c.571-8A>G, c.624_627delITCCT (p.D208fs), c.1211_1212delITG (p.L404fs), c.621T>G (p.D207E), c.1511G>A (p.R504H), c.1177C>T (p.R393X), c.2T>C (p.M11T), c.1292G>A (p.W431X), c.947_948insA (p.Y316fs), c.607T>G (p.W203G), c.1061_1063delITCT (p.F354_Y355delinsX), c.615delG (p.L205fs), c.805+2T>C, c.1123delG (p.E375fs), c.1121A>G (p.Q374R), c.1043_1046delITCAA (p.F348fs), c.1510delC (p.R504fs), c.1451T>C (p.L484P), c.964G>T (p.D322Y)

Usher Syndrome: Type 1F : Mutations (7): ♂ Genotyping | c.733C>T (p.R245X), c.2067C>A (p.Y684X), c.7C>T (p.R3X), c.1942C>T (p.R648X), c.1101delT (p.A367fsX), c.2800C>T (p.R934X), c.4272delA (p.L1425fs)

Usher Syndrome: Type 3 : Mutations (5): ♂ Genotyping | c.144T>G (p.N48K), c.131T>A (p.M120K), c.300T>G (p.Y176X), c.634C>T (p.Q212X), c.221T>C (p.L74P)

Walker-Warburg Syndrome : Mutations (1): ♂ Genotyping | c.1167insA (p.F390fs)

Residual Risk Information

Detection rates are calculated from the primary literature and may not be available for all ethnic populations. Sequencing detection rates and residual risks are reported as “greater than (>)” and “less than (<)” the values for genotyping, respectively, for each disease. More precise values may become available in the future.

Disease	Carrier Rate	Detection Rate	Residual Risk
Alpha Thalassemia	♂ General: 1/48	50.67%	1/97
Beta Thalassemia	♂ African American: 1/75	84.21%	1/475
	♂ Indian: 1/24	74.12%	1/93
	♂ Sardinians: 1/23	97.14%	1/804
	♂ Spaniard: 1/51	93.10%	1/739
Bloom Syndrome	♂ Ashkenazi Jewish: 1/134	96.67%	1/4,020
	♂ European: Unknown	66.22%	Unknown
	♂ Japanese: Unknown	50.00%	Unknown
Canavan Disease	♂ Ashkenazi Jewish: 1/55	98.86%	1/4,840
	♂ European: Unknown	53.23%	Unknown
Cystic Fibrosis	♂ African American: 1/62	69.99%	1/207
	♂ Ashkenazi Jewish: 1/23	96.81%	1/721
	♂ Asian: 1/94	66.40%	1/280
	♂ European: 1/25	94.96%	1/496
	♂ Hispanic American: 1/48	77.32%	1/212
	♂ Native American: 1/53	84.34%	1/338
Familial Dysautonomia	♂ Ashkenazi Jewish: 1/31	>99%	<1/3,100
Familial Hyperinsulinism: Type 1: ABCC8 Related	♂ Ashkenazi Jewish: 1/52	98.75%	1/4,160
	♂ Finnish: 1/101	45.16%	1/184
Fanconi Anemia: Type C	♂ Ashkenazi Jewish: 1/101	>99%	<1/10,100
	♂ General: Unknown	30.00%	Unknown
Gaucher Disease	♂ Ashkenazi Jewish: 1/15	87.16%	1/117
	♂ General: 1/112	31.60%	1/164
	♂ Spaniard: Unknown	44.29%	Unknown
	♂ Turkish: 1/236	59.38%	1/581
Glycogen Storage Disease: Type 1A	♂ Ashkenazi Jewish: 1/71	>99%	<1/7,100
	♂ Chinese: 1/159	80.00%	1/795
	♂ European: 1/177	76.88%	1/765
	♂ Hispanic American: 1/177	27.78%	1/245
	♂ Japanese: 1/177	89.22%	1/1,641
Joubert Syndrome	♂ Ashkenazi Jewish: 1/92	>99%	<1/9,200
Maple Syrup Urine Disease: Type 1B	♂ Ashkenazi Jewish: 1/97	>99%	<1/9,700
Maple Syrup Urine Disease: Type 3	♂ Ashkenazi Jewish: 1/94	>99%	<1/9,400
	♂ General: Unknown	68.75%	Unknown
Mucopolidosis: Type IV	♂ Ashkenazi Jewish: 1/97	96.15%	1/2,522
Nemaline Myopathy: NEB Related	♂ Ashkenazi Jewish: 1/108	>99%	<1/10,800
Niemann-Pick Disease: Type A	♂ Ashkenazi Jewish: 1/101	95.00%	1/2,020

Disease	Carrier Rate	Detection Rate	Residual Risk
Sickle-Cell Anemia	♂ African American: 1/10	>99%	<1/1,000
	♂ Hispanic American: 1/95	>99%	<1/9,500
Tay-Sachs Disease	♂ Argentinian: 1/280	82.35%	1/1,587
	♂ Ashkenazi Jewish: 1/29	99.53%	1/6,177
	♂ Cajun: 1/30	>99%	<1/3,000
	♂ European: 1/280	25.35%	1/375
	♂ General: 1/280	32.09%	1/412
	♂ Indian: Unknown	85.71%	Unknown
	♂ Iraqi Jewish: 1/140	56.25%	1/320
	♂ Japanese: 1/127	82.81%	1/739
	♂ Moroccan Jewish: 1/110	22.22%	1/141
	♂ Portuguese: 1/280	92.31%	1/3,640
	♂ Spaniard: 1/280	67.65%	1/865
	♂ United Kingdom: 1/161	71.43%	1/564
Usher Syndrome: Type 1F	♂ Ashkenazi Jewish: 1/126	93.75%	1/2,016
Usher Syndrome: Type 3	♂ Ashkenazi Jewish: 1/120	>99%	<1/12,000
	♂ Finnish: 1/134	>99%	<1/13,400
Walker-Warburg Syndrome	♂ Ashkenazi Jewish: 1/150	>99%	<1/15,000

Patient	Sample	Referring Doctor
Patient Name: Donor 4991 Date of Birth: [REDACTED] Reference #: P0386121 Indication: Carrier Testing Test Type: Unmask Additional Gene(s)	Specimen Type: Purified DNA (Semen) Lab #: [REDACTED] Date Collected: 4/26/2016 Date Received: 11/9/2018 Final Report: 11/27/2018	[REDACTED] Fairfax Cryobank, Inc. [REDACTED] [REDACTED] [REDACTED] Fax: [REDACTED]

RESULTS

A heterozygous (one copy) pathogenic variant, c.1222C>T, p.R408W, was detected in the *PAH* gene

Gene(s) analyzed: *PAH* (NM_000277.1)

Recommendations: Genetic counseling is recommended.

Interpretation: Screening for the presence of pathogenic variants in the *PAH* gene, which is associated with phenylalanine hydroxylase deficiency, was performed by next generation sequencing on DNA extracted from this patient's sample. A heterozygous (one copy) pathogenic missense variant, c.1222C>T, p.R408W, was detected in the *PAH* gene. When this variant is present in trans with a pathogenic variant, it is considered to be causative for phenylalanine hydroxylase deficiency. Therefore, this individual is expected to be at least a carrier for phenylalanine hydroxylase deficiency. Heterozygous carriers are not expected to exhibit symptoms of this disease.

No other variants of clinical interest were detected in this test.

What is phenylalanine hydroxylase deficiency?

Phenylalanine hydroxylase deficiency is an autosomal recessive disorder caused by pathogenic variants in the gene *PAH*. While it is found in many different ethnicities, it is particularly prevalent in Sephardic Jewish, Sicilian, Irish, and Turkish individuals, as well as Caucasians. Pathogenic *PAH* variants result in loss of function of the phenylalanine hydroxylase enzyme, which breaks down the amino acid phenylalanine. The most severe form of the disease is called phenylketonuria. If untreated, buildup of phenylalanine will result in irreversible brain damage and severe intellectual disability. Treatment involves the removal of phenylalanine from the diet. Even with strict adherence to the treatment, some neurologic deficiencies have been noticed in long-term survivors. Psychological problems, including anxiety, depression, phobias and panic attacks may occur in adults who do not comply well to their treatment. Some patients have a milder form of hyperphenylalaninemia and may tolerate higher levels of phenylalanine in their diet. Depending on the genotype, patients may be responsive to BH4, which can direct their treatment. However, it is not always possible to predict the severity of the disease based on genotype.

Patient: Donor 4991

DOB: [REDACTED]

Lab #: [REDACTED]

Comments:

This carrier screening test masks likely benign variants and variants of uncertain significance (VUS) if there are any. Only known pathogenic variants or likely pathogenic variants which are expected to result in deleterious effects on protein function are reported. If reporting of likely benign variants and VUS is desired in this patient, please contact the laboratory (tel. 212-241-2537) to request an amended report.

Please note these tests were developed and their performance characteristics were determined by Mount Sinai Genomics, Inc. They have not been cleared or approved by the FDA. These analyses generally provide highly accurate information regarding the patient's carrier or affected status. Despite this high level of accuracy, it should be kept in mind that there are many potential sources of diagnostic error, including misidentification of samples, polymorphisms, or other rare genetic variants that interfere with analysis. Families should understand that rare diagnostic errors may occur for these reasons.

This case has been reviewed and electronically signed by Ruth Kornreich, Ph.D., FACMG, Laboratory Director

Laboratory Medical Consultant: George A. Diaz, M.D., Ph.D.

Patient:	Donor 4991	DOB:	██████████	Lab #:	██████████
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Test Methods and Comments

Genomic DNA isolated from this patient was analyzed by one or more of the following methodologies, as applicable:

Fragile X CGG Repeat Analysis

PCR amplification using Asuragen, Inc. AmpliX[®] *FMR1* PCR reagents followed by capillary electrophoresis for allele sizing was performed. Samples positive for *FMR1* CGG repeats in the premutation and full mutation size range are further analyzed by Southern blot analysis to assess the size and methylation status of the *FMR1* CGG repeat.

Genotyping

Multiplex PCR amplification and allele specific primer extension analyses using the MassARRAY[®] System or Luminex[®] xMAP[®] technology were used to identify variants that are complex in nature or are present in low copy repeat regions and are, therefore, not amenable to Next Generation Sequencing technologies. Rare sequence variants may interfere with assay performance.

Multiplex Ligation-Dependent Probe Amplification (MLPA)

MLPA[®] probe sets and reagents, MRC-Holland, were used for the analysis of copy number of specific targets versus known control samples. Each target region was assayed with two adjacent oligonucleotide probes which following hybridization were ligated and used as template for subsequent rounds of amplification. Each complete probe within the assay has a unique length and amplicons are separated and identified by capillary electrophoresis. False positive or negative results may also occur due to rare sequence variants in target regions detected by MLPA probes. Analytical sensitivity and specificity of the MLPA method are both 99%.

For Alpha Thalassemia, the copy numbers of the *HBA1* and *HBA2* genes were analyzed. Alpha-globin gene deletions, triplications, and the Constant Spring (CS) mutation are assessed. However, it does not detect all known alpha-thalassemia mutations such as point mutations. In addition, carriers of alpha-thalassemia with three or more *HBA* copies on one chromosome, and one or no copies on the other chromosome, will not be detected. This test detects most alpha-globin gene deletions, triplications, and the Constant Spring (CS) mutation using Multiplex Ligation-Dependent Probe Amplification (MLPA). It is expected to detect approximately 90% of all alpha-thalassemia mutations, varying by ethnicity. Therefore, this result reduces, but does not eliminate, the chance that this patient is a carrier of alpha-thalassemia. With the exception of triplications, other benign alpha-globin gene polymorphisms will not be reported.

For Duchenne Muscular Dystrophy, the copy numbers of all *DMD* exons were analyzed. Please note that single-exon deletions and duplications will not be reported unless they are confirmed by NGS data (for example, if breakpoints occurring in an exon can be visualized).

For Spinal Muscular Atrophy (SMA), the copy numbers of the *SMN1* and *SMN2* genes were analyzed. The individual dosage of exons 7 and 8 as well as the combined dosage of exons 1, 4, 6 and 8 of *SMN1* and *SMN2* were assessed. Copy number gains and losses can be detected with this assay.

Depending on ethnicity 6 - 29 % of carriers will not be identified by dosage sensitive methods as this testing cannot detect individuals with two copies (duplication) of the *SMN1* gene on one chromosome and loss of *SMN1* (deletion) on the other chromosome (silent 2+0 carrier) or individuals that carry an intragenic mutation in *SMN1*. Please also note that 2% of individuals with SMA have an *SMN1* mutation that occurred *de novo*. Typically in these cases, only one parent is an SMA carrier.

Patient: Donor 4991	DOB: [REDACTED]	Lab #: [REDACTED]
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The presence of the g.27134T>G variant allele in an individual with Ashkenazi Jewish or Asian ancestry is indicative of a duplication of *SMN1*. When present in an Ashkenazi Jewish or Asian individual with two copies of *SMN1*, g.27134T>G is likely indicative of a silent (2+0) carrier. In individuals with two copies of *SMN1* with African American, Hispanic or Caucasian ancestry, the presence or absence of g.27134T>G significantly increases or decreases, respectively, the likelihood of being a silent 2+0 silent carrier.

Next Generation Sequencing (NGS)

NGS was performed on a panel of genes for the purpose of identifying pathogenic or likely pathogenic variants.

Agilent SureSelect™QXT technology was used with custom capture library to target the guaranteed list of mutations and exonic regions of the relevant genes. These targeted regions were sequenced using the Illumina HiSeq2500 system with 100 bp paired-end reads. The DNA sequences were mapped to and analyzed in comparison with the published human genome build UCSC hg19 reference sequence. The targeted coding exons and splice junctions of the known protein-coding RefSeq genes were assessed for the average depth of coverage and data quality threshold values. This technology may not detect all small insertion/deletions and is not diagnostic for large duplications/deletions, repeat expansions, and structural genomic variation. This test will detect variants within the exons and the intron-exon boundaries of the target regions. Variants outside these regions will either not be detected or are not guaranteed to be detected. These regions include, but are not limited to, UTRs, promoters, and deep intronic areas or regions that fall within low copy repeat segments. In addition, a mutation(s) in a gene not included on the panel could be present in this patient. All potentially pathogenic variants may be confirmed by either a specific genotyping assay or Sanger sequencing, if indicated. Any benign variants, likely benign variants or variants of uncertain significance identified during this analysis were not reported.

Sanger Sequencing

Sanger sequencing, as indicated, was performed in both directions using BigDye Terminator chemistry with the ABI 3730 DNA analyzer with target specific amplicons. It also may be used to supplement specific guaranteed target regions that fail NGS sequencing due to poor quality or low depth of coverage <20 reads or as a confirmatory method for NGS positive results. False negative results may occur if rare variants interfere with amplification or annealing.

Tay-Sachs Disease (TSD) Enzyme Analysis

Hexosaminidase activity and Hex A% activity were measured by a standard heat-inactivation, fluorometric method using artificial 4-MU-β-N-acetyl glucosaminide (4-MUG) substrate. This assay is highly sensitive and accurate in detecting Tay-Sachs carriers and individuals affected with TSD. It is estimated that less than 0.5% of Tay-Sachs carriers have non-carrier levels of percent Hex A activity, and therefore may not be identified by this assay. In addition, this assay may detect individuals that are carriers of or are affected with Sandhoff Disease. False positive results, such as pseudodeficiency alleles, may occur if benign variants interfere with the enzymatic assay. False negative results may occur if both *HEXA* and *HEXB* pathogenic variants are present in the same individual.

Please note these tests were developed and their performance characteristics were determined by Mount Sinai Genomics, Inc. They have not been cleared or approved by the FDA. These analyses generally provide highly accurate information regarding the patient's carrier or affected status. Despite this high level of accuracy, it should be kept in mind that there are many potential sources of diagnostic error, including misidentification of samples, polymorphisms, or other rare genetic variants that interfere with analysis. Families should understand that rare diagnostic errors may occur for these reasons.

SELECTED REFERENCES

Carrier Screening

Grody W et al. ACMG position statement on prenatal/preconception expanded carrier screening. *Genet Med*. 2013 15:482-

Patient: Donor 4991	DOB: [REDACTED]	Lab #: [REDACTED]
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Umbarger MA. Next-generation carrier screening. *Genet Med*. 2014 16:132-40.

Alpha-thalassemia:

Galanello R et al. Gene test review: Alpha-thalassemia. *Genet Med*. 2011 13:83-8.

Waye JS et al. Diagnostic testing for α -globin gene disorders in a heterogeneous North American population. *Int J Lab Hematol*. 2013 35:306-13.

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ACOG Committee Opinion. Number 325, Update on carrier screening for cystic fibrosis. 2005.

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Luo M et al. An Ashkenazi Jewish SMN1 haplotype specific to duplication alleles improves pan-ethnic carrier screening for spinal muscular atrophy. *Genet Med*. 2014 16:149-56.

Ashkenazi Jewish Disorders:

Scott SA et al. Experience with carrier screening and prenatal diagnosis for sixteen Ashkenazi Jewish Genetic Diseases. *Hum. Mutat*. 2010 31:1-11.

Duchenne Muscular Dystrophy:

Aartsma-Rus A et al. Entries in the Leiden Duchenne muscular dystrophy mutation database: an overview of mutation types and paradoxical cases that confirm the reading-frame rule. *Muscle Nerve*. 2006b 34:135-44.

Flanigan KM et al. Mutational spectrum of DMD mutations in dystrophinopathy patients: application of modern diagnostic techniques to a large cohort. *Hum Mutat*. 2009 30:1657-66.

Beta Globin-related Disorders:

Cao A et al. Beta-Thalassemia. GeneReviews (<http://www.ncbi.nlm.nih.gov/books/NBK1426/>)

Modell B et al. Epidemiology of haemoglobin disorders in Europe: an overview. *Scand J Clin Lab Invest*. 2007 67:39-69.

For further reading:

Orphanet: <http://www.orpha.net/consor/cgi-bin/index.php>

GeneReviews: <http://www.ncbi.nlm.nih.gov/books/NBK1116/>

For Disease Specific Standards and Guidelines:

<https://www.acmg.net/>

Additional disease-specific references available upon request.

Patient	Sample	Referring Doctor
Patient Name: Donor 4991 Date of Birth: [REDACTED] Reference #: P0386121 Indication: Carrier Testing Test Type: Unmask Additional Gene(s)	Specimen Type: Purified DNA (Semen) Lab #: [REDACTED] Date Collected: 4/26/2016 Date Received: 12/7/2018 Final Report: 12/27/2018	[REDACTED] Fairfax Cryobank, Inc. [REDACTED] [REDACTED] [REDACTED] Fax: [REDACTED]

RESULT SUMMARY

Negative: No clinically significant variant(s) detected

Gene(s) analyzed: *DHCR7* and *AGXT*

Recommendations:

Consideration of residual risk by ethnicity after a negative carrier screen is recommended, especially in the case of a positive family history for a specific disorder.

Interpretation:

Screening for the presence of pathogenic variants in the *DHCR7* and *AGXT* genes which are associated with Smith-Lemli-Opitz syndrome and primary hyperoxaluria, type 1, respectively, was performed by next generation sequencing and possibly genotyping for select variants on DNA extracted from this patient's sample. No clinically significant variants were detected during this analysis.

Please note that negative results reduce but do not eliminate the possibility that this individual is a carrier for the disorder(s) tested. Please see table of residual risks for specific detection rates and residual risk by ethnicity. With individuals of mixed ethnicity, it is recommended to use the highest residual risk estimate. Only variants determined to be pathogenic or likely pathogenic are reported in this carrier screening test.

Comments:

This carrier screening test masks likely benign variants and variants of uncertain significance (VUS) if there are any. Only known pathogenic variants or likely pathogenic variants which are expected to result in deleterious effects on protein function are reported. If reporting of likely benign variants and VUS is desired in this patient, please contact the laboratory (tel. 212-241-2537) to request an amended report.

Please note these tests were developed and their performance characteristics were determined by Mount Sinai Genomics, Inc. They have not been cleared or approved by the FDA. These analyses generally provide highly accurate information regarding the patient's carrier or affected status. Despite this high level of accuracy, it should be kept in mind that there are many potential sources of diagnostic error, including misidentification of samples, polymorphisms, or other rare genetic variants that interfere with analysis. Families should understand that rare diagnostic errors may occur for these reasons.

Patient: Donor 4991

DOB: [REDACTED]

Lab #: [REDACTED]

Table of Residual Risks by Ethnicity

Please note: This table displays residual risks after a negative result for each of the genes and corresponding disorders. **If a patient is reported to be a carrier of a disease, their residual risk is 1 and this table does not apply for that disease.**

Disease (Inheritance)	Gene	Ethnicity	Carrier Frequency	Detection Rate	Residual Risk	Analytical Detection Rate
Primary Hyperoxaluria, Type 1 (AR) NM_000030.2	AGXT	Worldwide	1 in 158	77%	1 in 684	>95%
Smith-Lemli-Opitz Syndrome (AR) NM_001360.2	DHCR7	Caucasian	1 in 48	81%	1 in 248	>95%
		African	1 in 93	67%	1 in 280	>95%
		Asian	< 1 in 500	84%	1 in 3120	>95%
		Ashkenazi Jewish	1 in 41	>95%	1 in 801	>95%
		Worldwide	1 in 68	80%	1 in 336	>95%

AR: Autosomal Recessive

This case has been reviewed and electronically signed by Ruth Kornreich, Ph.D., FACMG, Laboratory Director

Laboratory Medical Consultant: George A. Diaz, M.D., Ph.D.

Patient: Donor 4991	DOB: [REDACTED]	Lab #: [REDACTED]
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Test Methods and Comments

Genomic DNA isolated from this patient was analyzed by one or more of the following methodologies, as applicable:

Fragile X CGG Repeat Analysis

PCR amplification using Asuragen, Inc. AmpliX[®] *FMR1* PCR reagents followed by capillary electrophoresis for allele sizing was performed. Samples positive for *FMR1* CGG repeats in the premutation and full mutation size range are further analyzed by Southern blot analysis to assess the size and methylation status of the *FMR1* CGG repeat.

Genotyping

Multiplex PCR amplification and allele specific primer extension analyses using the MassARRAY[®] System or Luminex[®] xMAP[®] technology were used to identify variants that are complex in nature or are present in low copy repeat regions and are, therefore, not amenable to Next Generation Sequencing technologies. Rare sequence variants may interfere with assay performance.

Multiplex Ligation-Dependent Probe Amplification (MLPA)

MLPA[®] probe sets and reagents, MRC-Holland, were used for the analysis of copy number of specific targets versus known control samples. Each target region was assayed with two adjacent oligonucleotide probes which following hybridization were ligated and used as template for subsequent rounds of amplification. Each complete probe within the assay has a unique length and amplicons are separated and identified by capillary electrophoresis. False positive or negative results may also occur due to rare sequence variants in target regions detected by MLPA probes. Analytical sensitivity and specificity of the MLPA method are both 99%.

For Alpha Thalassemia, the copy numbers of the *HBA1* and *HBA2* genes were analyzed. Alpha-globin gene deletions, triplications, and the Constant Spring (CS) mutation are assessed. However, it does not detect all known alpha-thalassemia mutations such as point mutations. In addition, carriers of alpha-thalassemia with three or more *HBA* copies on one chromosome, and one or no copies on the other chromosome, will not be detected. This test detects most alpha-globin gene deletions, triplications, and the Constant Spring (CS) mutation using Multiplex Ligation-Dependent Probe Amplification (MLPA). It is expected to detect approximately 90% of all alpha-thalassemia mutations, varying by ethnicity. Therefore, this result reduces, but does not eliminate, the chance that this patient is a carrier of alpha-thalassemia. With the exception of triplications, other benign alpha-globin gene polymorphisms will not be reported.

For Duchenne Muscular Dystrophy, the copy numbers of all *DMD* exons were analyzed. Please note that single-exon deletions and duplications will not be reported unless they are confirmed by NGS data (for example, if breakpoints occurring in an exon can be visualized).

For Spinal Muscular Atrophy (SMA), the copy numbers of the *SMN1* and *SMN2* genes were analyzed. The individual dosage of exons 7 and 8 as well as the combined dosage of exons 1, 4, 6 and 8 of *SMN1* and *SMN2* were assessed. Copy number gains and losses can be detected with this assay.

Depending on ethnicity 6 - 29 % of carriers will not be identified by dosage sensitive methods as this testing cannot detect individuals with two copies (duplication) of the *SMN1* gene on one chromosome and loss of *SMN1* (deletion) on the other chromosome (silent 2+0 carrier) or individuals that carry an intragenic mutation in *SMN1*. Please also note that 2% of individuals with SMA have an *SMN1* mutation that occurred *de novo*. Typically in these cases, only one parent is an SMA carrier.

Patient: Donor 4991	DOB: [REDACTED]	Lab #: [REDACTED]
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The presence of the g.27134T>G variant allele in an individual with Ashkenazi Jewish or Asian ancestry is indicative of a duplication of *SMN1*. When present in an Ashkenazi Jewish or Asian individual with two copies of *SMN1*, g.27134T>G is likely indicative of a silent (2+0) carrier. In individuals with two copies of *SMN1* with African American, Hispanic or Caucasian ancestry, the presence or absence of g.27134T>G significantly increases or decreases, respectively, the likelihood of being a silent 2+0 silent carrier.

Next Generation Sequencing (NGS)

NGS was performed on a panel of genes for the purpose of identifying pathogenic or likely pathogenic variants.

Agilent SureSelect™QXT technology was used with custom capture library to target the guaranteed list of mutations and exonic regions of the relevant genes. These targeted regions were sequenced using the Illumina HiSeq2500 system with 100 bp paired-end reads. The DNA sequences were mapped to and analyzed in comparison with the published human genome build UCSC hg19 reference sequence. The targeted coding exons and splice junctions of the known protein-coding RefSeq genes were assessed for the average depth of coverage and data quality threshold values. This technology may not detect all small insertion/deletions and is not diagnostic for large duplications/deletions, repeat expansions, and structural genomic variation. This test will detect variants within the exons and the intron-exon boundaries of the target regions. Variants outside these regions will either not be detected or are not guaranteed to be detected. These regions include, but are not limited to, UTRs, promoters, and deep intronic areas or regions that fall within low copy repeat segments. In addition, a mutation(s) in a gene not included on the panel could be present in this patient. All potentially pathogenic variants may be confirmed by either a specific genotyping assay or Sanger sequencing, if indicated. Any benign variants, likely benign variants or variants of uncertain significance identified during this analysis were not reported.

Sanger Sequencing

Sanger sequencing, as indicated, was performed in both directions using BigDye Terminator chemistry with the ABI 3730 DNA analyzer with target specific amplicons. It also may be used to supplement specific guaranteed target regions that fail NGS sequencing due to poor quality or low depth of coverage <20 reads or as a confirmatory method for NGS positive results. False negative results may occur if rare variants interfere with amplification or annealing.

Tay-Sachs Disease (TSD) Enzyme Analysis

Hexosaminidase activity and Hex A% activity were measured by a standard heat-inactivation, fluorometric method using artificial 4-MU-β-N-acetyl glucosaminide (4-MUG) substrate. This assay is highly sensitive and accurate in detecting Tay-Sachs carriers and individuals affected with TSD. It is estimated that less than 0.5% of Tay-Sachs carriers have non-carrier levels of percent Hex A activity, and therefore may not be identified by this assay. In addition, this assay may detect individuals that are carriers of or are affected with Sandhoff Disease. False positive results, such as pseudodeficiency alleles, may occur if benign variants interfere with the enzymatic assay. False negative results may occur if both *HEXA* and *HEXB* pathogenic variants are present in the same individual.

Please note these tests were developed and their performance characteristics were determined by Mount Sinai Genomics, Inc. They have not been cleared or approved by the FDA. These analyses generally provide highly accurate information regarding the patient's carrier or affected status. Despite this high level of accuracy, it should be kept in mind that there are many potential sources of diagnostic error, including misidentification of samples, polymorphisms, or other rare genetic variants that interfere with analysis. Families should understand that rare diagnostic errors may occur for these reasons.

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Patient: Donor 4991	DOB: [REDACTED]	Lab #: [REDACTED]
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Luo M et al. An Ashkenazi Jewish SMN1 haplotype specific to duplication alleles improves pan-ethnic carrier screening for spinal muscular atrophy. *Genet Med*. 2014 16:149-56.

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Flanigan KM et al. Mutational spectrum of DMD mutations in dystrophinopathy patients: application of modern diagnostic techniques to a large cohort. *Hum Mutat*. 2009 30:1657-66.

Beta Globin-related Disorders:

Cao A et al. Beta-Thalassemia. GeneReviews (<http://www.ncbi.nlm.nih.gov/books/NBK1426/>)

Modell B et al. Epidemiology of haemoglobin disorders in Europe: an overview. *Scand J Clin Lab Invest*. 2007 67:39-69.

For further reading:

Orphanet: <http://www.orpha.net/consor/cgi-bin/index.php>

GeneReviews: <http://www.ncbi.nlm.nih.gov/books/NBK1116/>

For Disease Specific Standards and Guidelines:

<https://www.acmg.net/>

Additional disease-specific references available upon request.

Patient	Sample	Referring Doctor
Patient Name: Donor 4991 Date of Birth: [REDACTED] Reference #: FFAXCB-S44991 Indication: Carrier Testing Test Type: Unmask Additional Gene(s)	Specimen Type: Purified DNA (semen) Lab #: [REDACTED] Date Collected: 2/4/2019 Date Received: 2/11/2019 Final Report: 2/25/2019	[REDACTED] Fairfax Cryobank, Inc. [REDACTED] [REDACTED] [REDACTED] [REDACTED]

RESULTS

Negative: No clinically significant variant(s) detected

Gene(s) analyzed: *DNAI2*

Recommendations:

Consideration of residual risk by ethnicity after a negative carrier screen is recommended, especially in the case of a positive family history for a specific disorder.

Interpretation:

Screening for the presence of pathogenic variants in the *DNAI2* gene which is associated with primary ciliary dyskinesia (*DNAI2*-related) was performed by next generation sequencing and possibly genotyping for select variants on DNA extracted from this patient's sample. No clinically significant variants were detected during this analysis.

Please note that negative results reduce but do not eliminate the possibility that this individual is a carrier for the disorder(s) tested. Please see table of residual risks for specific detection rates and residual risk by ethnicity. With individuals of mixed ethnicity, it is recommended to use the highest residual risk estimate. Only variants determined to be pathogenic or likely pathogenic are reported in this carrier screening test.

Comments:

This carrier screening test masks likely benign variants and variants of uncertain significance (VUS) if there are any. Only known pathogenic variants or likely pathogenic variants which are expected to result in deleterious effects on protein function are reported. If reporting of likely benign variants and VUS is desired in this patient, please contact the laboratory (tel. 212-241-2537) to request an amended report.

Please note these tests were developed and their performance characteristics were determined by Mount Sinai Genomics, Inc. They have not been cleared or approved by the FDA. These analyses generally provide highly accurate information regarding the patient's carrier or affected status. Despite this high level of accuracy, it should be kept in mind that there are many potential sources of diagnostic error, including misidentification of samples, polymorphisms, or other rare genetic variants that interfere with analysis. Families should understand that rare diagnostic errors may occur for these reasons.

Patient:	Donor 4991	DOB:	██████	Lab #:	██████████
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Table of Residual Risks by Ethnicity

Please note: This table displays residual risks after a negative result for each of the genes and corresponding disorders. **If a patient is reported to be a carrier of a disease, their residual risk is 1 and this table does not apply for that disease.**

Disease (Inheritance)	Gene	Ethnicity	Carrier Frequency	Detection Rate	Residual Risk	Analytical Detection Rate
Primary Ciliary Dyskinesia (<i>DNAI2</i> -Related) (AR) NM_023036.4	<i>DNAI2</i>	Ashkenazi Jewish	1 in 200	>95%	1 in 3981	>95%
		Worldwide	1 in 500	>95%	1 in 9981	>95%

AR: Autosomal Recessive

This case has been reviewed and electronically signed by Ruth Kornreich, Ph.D., FACMG, Laboratory Director

Laboratory Medical Consultant: George A. Diaz, M.D., Ph.D.

Patient:	Donor 4991	DOB:	██████████	Lab #:	██████████
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Test Methods and Comments

Genomic DNA isolated from this patient was analyzed by one or more of the following methodologies, as applicable:

Fragile X CGG Repeat Analysis

PCR amplification using Asuragen, Inc. AmpliX[®] *FMR1* PCR reagents followed by capillary electrophoresis for allele sizing was performed. Samples positive for *FMR1* CGG repeats in the premutation and full mutation size range are further analyzed by Southern blot analysis to assess the size and methylation status of the *FMR1* CGG repeat.

Genotyping

Multiplex PCR amplification and allele specific primer extension analyses using the MassARRAY[®] System or Luminex[®] xMAP[®] technology were used to identify variants that are complex in nature or are present in low copy repeat regions and are, therefore, not amenable to Next Generation Sequencing technologies. Rare sequence variants may interfere with assay performance.

Multiplex Ligation-Dependent Probe Amplification (MLPA)

MLPA[®] probe sets and reagents, MRC-Holland, were used for the analysis of copy number of specific targets versus known control samples. Each target region was assayed with two adjacent oligonucleotide probes which following hybridization were ligated and used as template for subsequent rounds of amplification. Each complete probe within the assay has a unique length and amplicons are separated and identified by capillary electrophoresis. False positive or negative results may also occur due to rare sequence variants in target regions detected by MLPA probes. Analytical sensitivity and specificity of the MLPA method are both 99%.

For Alpha Thalassemia, the copy numbers of the *HBA1* and *HBA2* genes were analyzed. Alpha-globin gene deletions, triplications, and the Constant Spring (CS) mutation are assessed. However, it does not detect all known alpha-thalassemia mutations such as point mutations. In addition, carriers of alpha-thalassemia with three or more *HBA* copies on one chromosome, and one or no copies on the other chromosome, will not be detected. This test detects most alpha-globin gene deletions, triplications, and the Constant Spring (CS) mutation using Multiplex Ligation-Dependent Probe Amplification (MLPA). It is expected to detect approximately 90% of all alpha-thalassemia mutations, varying by ethnicity. Therefore, this result reduces, but does not eliminate, the chance that this patient is a carrier of alpha-thalassemia. With the exception of triplications, other benign alpha-globin gene polymorphisms will not be reported.

For Duchenne Muscular Dystrophy, the copy numbers of all *DMD* exons were analyzed. Please note that single-exon deletions and duplications will not be reported unless they are confirmed by NGS data (for example, if breakpoints occurring in an exon can be visualized).

For Spinal Muscular Atrophy (SMA), the copy numbers of the *SMN1* and *SMN2* genes were analyzed. The individual dosage of exons 7 and 8 as well as the combined dosage of exons 1, 4, 6 and 8 of *SMN1* and *SMN2* were assessed. Copy number gains and losses can be detected with this assay.

Depending on ethnicity 6 - 29 % of carriers will not be identified by dosage sensitive methods as this testing cannot detect individuals with two copies (duplication) of the *SMN1* gene on one chromosome and loss of *SMN1* (deletion) on the other chromosome (silent 2+0 carrier) or individuals that carry an intragenic mutation in *SMN1*. Please also note that 2% of individuals with SMA have an *SMN1* mutation that occurred *de novo*. Typically in these cases, only one parent is an SMA carrier.

Patient: Donor 4991	DOB: [REDACTED]	Lab #: [REDACTED]
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The presence of the g.27134T>G variant allele in an individual with Ashkenazi Jewish or Asian ancestry is indicative of a duplication of *SMN1*. When present in an Ashkenazi Jewish or Asian individual with two copies of *SMN1*, g.27134T>G is likely indicative of a silent (2+0) carrier. In individuals with two copies of *SMN1* with African American, Hispanic or Caucasian ancestry, the presence or absence of g.27134T>G significantly increases or decreases, respectively, the likelihood of being a silent 2+0 silent carrier.

Next Generation Sequencing (NGS)

NGS was performed on a panel of genes for the purpose of identifying pathogenic or likely pathogenic variants.

Agilent SureSelect™QXT technology was used with custom capture library to target the guaranteed list of mutations and exonic regions of the relevant genes. These targeted regions were sequenced using the Illumina HiSeq2500 system with 100 bp paired-end reads. The DNA sequences were mapped to and analyzed in comparison with the published human genome build UCSC hg19 reference sequence. The targeted coding exons and splice junctions of the known protein-coding RefSeq genes were assessed for the average depth of coverage and data quality threshold values. This technology may not detect all small insertion/deletions and is not diagnostic for large duplications/deletions, repeat expansions, and structural genomic variation. This test will detect variants within the exons and the intron-exon boundaries of the target regions. Variants outside these regions will either not be detected or are not guaranteed to be detected. These regions include, but are not limited to, UTRs, promoters, and deep intronic areas or regions that fall within low copy repeat segments. In addition, a mutation(s) in a gene not included on the panel could be present in this patient. All potentially pathogenic variants may be confirmed by either a specific genotyping assay or Sanger sequencing, if indicated. Any benign variants, likely benign variants or variants of uncertain significance identified during this analysis were not reported.

Sanger Sequencing

Sanger sequencing, as indicated, was performed in both directions using BigDye Terminator chemistry with the ABI 3730 DNA analyzer with target specific amplicons. It also may be used to supplement specific guaranteed target regions that fail NGS sequencing due to poor quality or low depth of coverage <20 reads or as a confirmatory method for NGS positive results. False negative results may occur if rare variants interfere with amplification or annealing.

Tay-Sachs Disease (TSD) Enzyme Analysis

Hexosaminidase activity and Hex A% activity were measured by a standard heat-inactivation, fluorometric method using artificial 4-MU-β-N-acetyl glucosaminide (4-MUG) substrate. This assay is highly sensitive and accurate in detecting Tay-Sachs carriers and individuals affected with TSD. It is estimated that less than 0.5% of Tay-Sachs carriers have non-carrier levels of percent Hex A activity, and therefore may not be identified by this assay. In addition, this assay may detect individuals that are carriers of or are affected with Sandhoff Disease. False positive results, such as pseudodeficiency alleles, may occur if benign variants interfere with the enzymatic assay. False negative results may occur if both *HEXA* and *HEXB* pathogenic variants are present in the same individual.

Please note these tests were developed and their performance characteristics were determined by Mount Sinai Genomics, Inc. They have not been cleared or approved by the FDA. These analyses generally provide highly accurate information regarding the patient's carrier or affected status. Despite this high level of accuracy, it should be kept in mind that there are many potential sources of diagnostic error, including misidentification of samples, polymorphisms, or other rare genetic variants that interfere with analysis. Families should understand that rare diagnostic errors may occur for these reasons.

SELECTED REFERENCES

Carrier Screening

Grody W et al. ACMG position statement on prenatal/preconception expanded carrier screening. *Genet Med*. 2013 15:482-

Patient: Donor 4991	DOB: [REDACTED]	Lab #: [REDACTED]
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3.

Umbarger MA. Next-generation carrier screening. *Genet Med*. 2014 16:132-40.

Alpha-thalassemia:

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Waye JS et al. Diagnostic testing for α -globin gene disorders in a heterogeneous North American population. *Int J Lab Hematol*. 2013 35:306-13.

Cystic Fibrosis:

ACOG Committee Opinion. Number 325, Update on carrier screening for cystic fibrosis. 2005.

Fragile X syndrome:

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Spinal Muscular Atrophy:

Hendrickson BC et al. Differences in SMN1 allele frequencies among ethnic groups within North America. *J Med Genet*. 2009 46:641-4.

Ogino S et al. Genetic risk assessment in carrier testing for spinal muscular atrophy. *Am J Med Genet*. 2002 110:301-7.

Luo M et al. An Ashkenazi Jewish SMN1 haplotype specific to duplication alleles improves pan-ethnic carrier screening for spinal muscular atrophy. *Genet Med*. 2014 16:149-56.

Ashkenazi Jewish Disorders:

Scott SA et al. Experience with carrier screening and prenatal diagnosis for sixteen Ashkenazi Jewish Genetic Diseases. *Hum. Mutat*. 2010 31:1-11.

Duchenne Muscular Dystrophy:

Aartsma-Rus A et al. Entries in the Leiden Duchenne muscular dystrophy mutation database: an overview of mutation types and paradoxical cases that confirm the reading-frame rule. *Muscle Nerve*. 2006b 34:135-44.

Flanigan KM et al. Mutational spectrum of DMD mutations in dystrophinopathy patients: application of modern diagnostic techniques to a large cohort. *Hum Mutat*. 2009 30:1657-66.

Beta Globin-related Disorders:

Cao A et al. Beta-Thalassemia. GeneReviews (<http://www.ncbi.nlm.nih.gov/books/NBK1426/>)

Modell B et al. Epidemiology of haemoglobin disorders in Europe: an overview. *Scand J Clin Lab Invest*. 2007 67:39-69.

For further reading:

Orphanet: <http://www.orpha.net/consor/cgi-bin/index.php>

GeneReviews: <http://www.ncbi.nlm.nih.gov/books/NBK1116/>

For Disease Specific Standards and Guidelines:

<https://www.acmg.net/>

Additional disease-specific references available upon request.

Patient	Sample	Referring Doctor
Patient Name: Donor 4991 Date of Birth: [REDACTED] Reference #: FFAXCB-S44991 Indication: Carrier Testing Test Type: Unmask Additional Gene(s)	Specimen Type: Purified DNA semen Lab #: [REDACTED] Date Collected: 4/26/2016 Date Received: 4/1/2019 Final Report: 4/15/2019	[REDACTED] Fairfax Cryobank, Inc. [REDACTED] [REDACTED] [REDACTED] [REDACTED]

RESULT SUMMARY

Negative: No clinically significant variant(s) detected

Gene(s) analyzed: *BTBD*

Recommendations:

Consideration of residual risk by ethnicity after a negative carrier screen is recommended, especially in the case of a positive family history for a specific disorder.

Interpretation:

Screening for the presence of pathogenic variants in the *BTD* gene which is associated with biotinidase deficiency was performed by next generation sequencing and possibly genotyping for select variants on DNA extracted from this patient's sample. No clinically significant variants were detected during this analysis.

Please note that negative results reduce but do not eliminate the possibility that this individual is a carrier for the disorder(s) tested. Please see table of residual risks for specific detection rates and residual risk by ethnicity. With individuals of mixed ethnicity, it is recommended to use the highest residual risk estimate. Only variants determined to be pathogenic or likely pathogenic are reported in this carrier screening test.

Comments:

This carrier screening test masks likely benign variants and variants of uncertain significance (VUS) if there are any. Only known pathogenic variants or likely pathogenic variants which are expected to result in deleterious effects on protein function are reported. If reporting of likely benign variants and VUS is desired in this patient, please contact the laboratory (tel. 212-241-2537) to request an amended report.

Please note these tests were developed and their performance characteristics were determined by Mount Sinai Genomics, Inc. They have not been cleared or approved by the FDA. These analyses generally provide highly accurate information regarding the patient's carrier or affected status. Despite this high level of accuracy, it should be kept in mind that there are many potential sources of diagnostic error, including misidentification of samples, polymorphisms, or other rare genetic variants that interfere with analysis. Families should understand that rare diagnostic errors may occur for these reasons.

Patient: Donor 4991

DOB: [REDACTED]

Lab #: [REDACTED]

Table of Residual Risks by Ethnicity

Please note: This table displays residual risks after a negative result for each of the genes and corresponding disorders. **If a patient is reported to be a carrier of a disease, their residual risk is 1 and this table does not apply for that disease.**

Disease (Inheritance)	Gene	Ethnicity	Carrier Frequency	Detection Rate	Residual Risk	Analytical Detection Rate
Biotinidase Deficiency (AR) NM_000060.3	BTB	Caucasian	1 in 12	80%	1 in 56	86%
		Hispanic	1 in 30	72%	1 in 105	>95%
		Worldwide	1 in 25	74%	1 in 93	>95%

AR: Autosomal Recessive

This case has been reviewed and electronically signed by Ruth Kornreich, Ph.D., FACMG, Laboratory Director

Laboratory Medical Consultant: George A. Diaz, M.D., Ph.D.

Patient: Donor 4991	DOB: [REDACTED]	Lab #: [REDACTED]
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Test Methods and Comments

Genomic DNA isolated from this patient was analyzed by one or more of the following methodologies, as applicable:

Fragile X CGG Repeat Analysis

PCR amplification using Asuragen, Inc. AmpliX[®] *FMR1* PCR reagents followed by capillary electrophoresis for allele sizing was performed. Samples positive for *FMR1* CGG repeats in the premutation and full mutation size range are further analyzed by Southern blot analysis to assess the size and methylation status of the *FMR1* CGG repeat.

Genotyping

Multiplex PCR amplification and allele specific primer extension analyses using the MassARRAY[®] System or Luminex[®] xMAP[®] technology were used to identify variants that are complex in nature or are present in low copy repeat regions and are, therefore, not amenable to Next Generation Sequencing technologies. Rare sequence variants may interfere with assay performance.

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For Alpha Thalassemia, the copy numbers of the *HBA1* and *HBA2* genes were analyzed. Alpha-globin gene deletions, triplications, and the Constant Spring (CS) mutation are assessed. However, it does not detect all known alpha-thalassemia mutations such as point mutations. In addition, carriers of alpha-thalassemia with three or more *HBA* copies on one chromosome, and one or no copies on the other chromosome, will not be detected. This test detects most alpha-globin gene deletions, triplications, and the Constant Spring (CS) mutation using Multiplex Ligation-Dependent Probe Amplification (MLPA). It is expected to detect approximately 90% of all alpha-thalassemia mutations, varying by ethnicity. Therefore, this result reduces, but does not eliminate, the chance that this patient is a carrier of alpha-thalassemia. With the exception of triplications, other benign alpha-globin gene polymorphisms will not be reported.

For Duchenne Muscular Dystrophy, the copy numbers of all *DMD* exons were analyzed. Please note that single-exon deletions and duplications will not be reported unless they are confirmed by NGS data (for example, if breakpoints occurring in an exon can be visualized).

For Spinal Muscular Atrophy (SMA), the copy numbers of the *SMN1* and *SMN2* genes were analyzed. The individual dosage of exons 7 and 8 as well as the combined dosage of exons 1, 4, 6 and 8 of *SMN1* and *SMN2* were assessed. Copy number gains and losses can be detected with this assay.

Depending on ethnicity 6 - 29 % of carriers will not be identified by dosage sensitive methods as this testing cannot detect individuals with two copies (duplication) of the *SMN1* gene on one chromosome and loss of *SMN1* (deletion) on the other chromosome (silent 2+0 carrier) or individuals that carry an intragenic mutation in *SMN1*. Please also note that 2% of individuals with SMA have an *SMN1* mutation that occurred *de novo*. Typically in these cases, only one parent is an SMA carrier.

Patient: Donor 4991	DOB: [REDACTED]	Lab #: [REDACTED]
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The presence of the g.27134T>G variant allele in an individual with Ashkenazi Jewish or Asian ancestry is indicative of a duplication of *SMN1*. When present in an Ashkenazi Jewish or Asian individual with two copies of *SMN1*, g.27134T>G is likely indicative of a silent (2+0) carrier. In individuals with two copies of *SMN1* with African American, Hispanic or Caucasian ancestry, the presence or absence of g.27134T>G significantly increases or decreases, respectively, the likelihood of being a silent 2+0 silent carrier.

Next Generation Sequencing (NGS)

NGS was performed on a panel of genes for the purpose of identifying pathogenic or likely pathogenic variants.

Agilent SureSelect™QXT technology was used with custom capture library to target the guaranteed list of mutations and exonic regions of the relevant genes. These targeted regions were sequenced using the Illumina HiSeq2500 system with 100 bp paired-end reads. The DNA sequences were mapped to and analyzed in comparison with the published human genome build UCSC hg19 reference sequence. The targeted coding exons and splice junctions of the known protein-coding RefSeq genes were assessed for the average depth of coverage and data quality threshold values. This technology may not detect all small insertion/deletions and is not diagnostic for large duplications/deletions, repeat expansions, and structural genomic variation. This test will detect variants within the exons and the intron-exon boundaries of the target regions. Variants outside these regions will either not be detected or are not guaranteed to be detected. These regions include, but are not limited to, UTRs, promoters, and deep intronic areas or regions that fall within low copy repeat segments. In addition, a mutation(s) in a gene not included on the panel could be present in this patient. All potentially pathogenic variants may be confirmed by either a specific genotyping assay or Sanger sequencing, if indicated. Any benign variants, likely benign variants or variants of uncertain significance identified during this analysis were not reported.

Sanger Sequencing

Sanger sequencing, as indicated, was performed in both directions using BigDye Terminator chemistry with the ABI 3730 DNA analyzer with target specific amplicons. It also may be used to supplement specific guaranteed target regions that fail NGS sequencing due to poor quality or low depth of coverage <20 reads or as a confirmatory method for NGS positive results. False negative results may occur if rare variants interfere with amplification or annealing.

Tay-Sachs Disease (TSD) Enzyme Analysis

Hexosaminidase activity and Hex A% activity were measured by a standard heat-inactivation, fluorometric method using artificial 4-MU-β-N-acetyl glucosaminide (4-MUG) substrate. This assay is highly sensitive and accurate in detecting Tay-Sachs carriers and individuals affected with TSD. It is estimated that less than 0.5% of Tay-Sachs carriers have non-carrier levels of percent Hex A activity, and therefore may not be identified by this assay. In addition, this assay may detect individuals that are carriers of or are affected with Sandhoff Disease. False positive results, such as pseudodeficiency alleles, may occur if benign variants interfere with the enzymatic assay. False negative results may occur if both *HEXA* and *HEXB* pathogenic variants are present in the same individual.

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SELECTED REFERENCES

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Patient: Donor 4991	DOB: [REDACTED]	Lab #: [REDACTED]
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Spinal Muscular Atrophy:

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Ogino S et al. Genetic risk assessment in carrier testing for spinal muscular atrophy. *Am J Med Genet*. 2002 110:301-7.

Luo M et al. An Ashkenazi Jewish SMN1 haplotype specific to duplication alleles improves pan-ethnic carrier screening for spinal muscular atrophy. *Genet Med*. 2014 16:149-56.

Ashkenazi Jewish Disorders:

Scott SA et al. Experience with carrier screening and prenatal diagnosis for sixteen Ashkenazi Jewish Genetic Diseases. *Hum. Mutat*. 2010 31:1-11.

Duchenne Muscular Dystrophy:

Aartsma-Rus A et al. Entries in the Leiden Duchenne muscular dystrophy mutation database: an overview of mutation types and paradoxical cases that confirm the reading-frame rule. *Muscle Nerve*. 2006b 34:135-44.

Flanigan KM et al. Mutational spectrum of DMD mutations in dystrophinopathy patients: application of modern diagnostic techniques to a large cohort. *Hum Mutat*. 2009 30:1657-66.

Beta Globin-related Disorders:

Cao A et al. Beta-Thalassemia. GeneReviews (<http://www.ncbi.nlm.nih.gov/books/NBK1426/>)

Modell B et al. Epidemiology of haemoglobin disorders in Europe: an overview. *Scand J Clin Lab Invest*. 2007 67:39-69.

For further reading:

Orphanet: <http://www.orpha.net/consor/cgi-bin/index.php>

GeneReviews: <http://www.ncbi.nlm.nih.gov/books/NBK1116/>

For Disease Specific Standards and Guidelines:

<https://www.acmg.net/>

Additional disease-specific references available upon request.

Patient	Sample	Referring Doctor
Patient Name: Donor 4991 Date of Birth: [REDACTED] Reference #: FFAXCB-S44991 Indication: Carrier Testing Test Type: Congenital Adrenal Hyperplasia (CYP21A2) Carrier Screen	Specimen Type: Purified DNA Lab #: [REDACTED] Date Collected: 4/5/2019 Date Received: 4/6/2019 Final Report: 4/28/2019	[REDACTED] Fairfax Cryobank, Inc. [REDACTED] [REDACTED] [REDACTED] [REDACTED]

RESULTS

NEGATIVE for congenital adrenal hyperplasia (due to 21-hydroxylase deficiency)

CYP21A2 copy number: 2

No pathogenic copy number variants detected

No pathogenic sequence variants detected in CYP21A2

Reduced risk of being a congenital adrenal hyperplasia carrier

Genes analyzed: CYP21A2 (NM_000500.6)

Inheritance: Autosomal Recessive

Recommendations

Consideration of residual risk by ethnicity (see below) after a negative carrier screen is recommended, especially in the case of a positive family history of congenital adrenal hyperplasia.

Interpretation

This individual was negative for all pathogenic CYP21A2 copy number variants that were tested, and no pathogenic or likely pathogenic variants were identified by sequence analysis. These negative results reduce but do not eliminate the possibility that this individual is a carrier. See *Table of Residual Risks Based on Ethnicity*. With individuals of mixed ethnicity, it is recommended to use the highest residual risk estimate.

Table of Residual Risk Based On Ethnicity - Classic Congenital Adrenal Hyperplasia Due to 21-Hydroxylase Deficiency

Ethnicity	Carrier Frequency	Detection Rate	Residual Risk
Ashkenazi Jewish	1 in 40	>95%	1 in 780
Caucasian	1 in 67	>95%	1 in 1300
Worldwide	1 in 60	>95%	1 in 1200

Patient: Donor 4991

DOB: [REDACTED]

Lab #: [REDACTED]

Table of Residual Risk Based On Ethnicity - Non-Classic Congenital Adrenal Hyperplasia Due to 21-Hydroxylase Deficiency

Ethnicity	Carrier Frequency	Detection Rate	Residual Risk
Ashkenazi Jewish	1 in 7	>95%	1 in 120
Caucasian	1 in 11	>95%	1 in 200
Worldwide	1 in 16	>95%	1 in 300

Test Methods and Comments

Genomic DNA isolated from this patient was analyzed by one or more of the following methodologies, as applicable:

Multiplex Ligation-Dependent Probe Amplification (MLPA) (Analytical Detection Rate >99%)

MLPA® probe sets and reagents from MRC-Holland were used for copy number analysis of specific targets versus known control samples. False positive or negative results may occur due to rare sequence variants in target regions detected by MLPA probes. Analytical sensitivity and specificity of the MLPA method are both 99%.

For congenital adrenal hyperplasia, the copy number of the *CYP21A2* gene was analyzed. This analysis can detect large deletions due to unequal meiotic crossing-over between *CYP21A2* and the pseudogene *CYP21A1P*. These 30-kb deletions make up approximately 20% of *CYP21A2* pathogenic alleles. This test may also identify certain point mutations in *CYP21A2* caused by gene conversion events between *CYP21A2* and *CYP21A1P*. Some carriers may not be identified by dosage sensitive methods as this testing cannot detect individuals with two copies (duplication) of the *CYP21A2* gene on one chromosome and loss of *CYP21A2* (deletion) on the other chromosome. Analysis of *CYP21A2* is performed in association with long-range PCR of the coding regions followed by short-read sequencing.

Long-Range PCR (Analytical Detection Rate >99%)

A long-range PCR was performed to generate a locus-specific amplicon for *CYP21A2*. The PCR product was then prepared for short-read NGS sequencing as described below and sequenced. Sequenced reads were mapped back to the original genomic loci and converted to VCF files as described below.

A certain percentage of healthy individuals carry a duplication of the *CYP21A2* gene, which has no clinical consequences. In cases where two copies of a gene are located on the same chromosome in tandem, only the second copy will be amplified and assessed for potentially pathogenic variants, due to size limitations of the PCR reaction. However, because these alleles contain at least two copies of the *CYP21A2* gene in tandem, it is expected that this patient has at least one functional gene in the tandem allele and this patient is therefore less likely to be a carrier. When an individual carries both a duplication allele and a pathogenic variant, or multiple pathogenic variants, the current analysis may not be able to determine the phase (cis/trans configuration) of the *CYP21A2* alleles identified. Family studies may be required in certain scenarios where phasing is required to determine the carrier status.

Next Generation Sequencing (NGS) (Analytical Detection Rate >95%)

Samples sequenced on the Illumina HiSeq 2500 platform in the Rapid Run mode or the Illumina NovaSeq platform in the Xp workflow, using 100 bp paired-end reads. The sequencing data was analyzed using a custom bioinformatics algorithm designed and validated in house.

Patient: Donor 4991

DOB: [REDACTED]

Lab #: [REDACTED]

The coding exons and splice junctions of the tested genes were assessed for the average depth of coverage (minimum of 20X) and data quality threshold values. Exons not meeting a minimum of >20X read depth across the exon are further analyzed by Sanger sequencing.

This test will detect variants within the exons and the intron-exon boundaries of the target regions. Variants outside these regions may not be detected, including, but not limited to, UTRs, promoters, and deep intronic areas. This technology may not detect all small insertion/deletions and is not diagnostic for repeat expansions and structural genomic variation. In addition, a mutation(s) in a gene not included on the panel could be present in this patient.

Variant interpretation and classification was performed based on the American College of Medical Genetics Standards and Guidelines for the Interpretation of Sequence Variants (Richards et al, 2015). All potentially pathogenic variants may be confirmed by either a specific genotyping assay or Sanger sequencing, if indicated. Any benign variants, likely benign variants or variants of uncertain significance identified during this analysis will not be reported.

Variant interpretation and classification was performed based on the American College of Medical Genetics Standards and Guidelines for the Interpretation of Sequence Variants (Richards et al, 2015). All potentially pathogenic variants may be confirmed by either a specific genotyping assay or Sanger sequencing, if indicated. Any benign variants, likely benign variants or variants of uncertain significance identified during this analysis will not be reported.

Sanger Sequencing (Confirmation method) (Accuracy >99%)

Sanger sequencing, as indicated, was performed using BigDye Terminator chemistry with the ABI 3730 DNA analyzer with target specific amplicons. It also may be used to supplement specific guaranteed target regions that fail NGS sequencing due to poor quality or low depth of coverage (<20 reads) or as a confirmatory method for NGS positive results. False negative results may occur if rare variants interfere with amplification or annealing.

Please note these tests were developed and their performance characteristics were determined by Mount Sinai Genomics, Inc. They have not been cleared or approved by the FDA. These analyses generally provide highly accurate information regarding the patient's carrier or affected status. Despite this high level of accuracy, it should be kept in mind that there are many potential sources of diagnostic error, including misidentification of samples, polymorphisms, or other rare genetic variants that interfere with analysis. Families should understand that rare diagnostic errors may occur for these reasons.

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Grody W et al. ACMG position statement on prenatal/preconception expanded carrier screening. *Genet Med*. 2013 15:482-3.

Variant Classification:

Richards S et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015 May;17(5):405-24

Additional disease-specific references available upon request.

This case has been reviewed and electronically signed by Ruth Kornreich, Ph.D., FACMG, Laboratory Director

Laboratory Medical Consultant: George A. Diaz, M.D., Ph.D.

Patient: Donor 4991	DOB: 9/1/1986	Lab #: [REDACTED]
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Patient	Sample	Referring Doctor
Patient Name: Donor 4991 Date of Birth: [REDACTED] Reference #: [REDACTED] Indication: Carrier Testing Test Type: Unmask Additional Gene(s)	Specimen Type: Purified DNA (Semen) Lab #: 17074881UDDD Date Collected: 4/26/2016 Date Received: 8/14/2022 Final Report: 9/7/2022	[REDACTED] Fairfax Cryobank, Inc. [REDACTED] [REDACTED] [REDACTED] [REDACTED]

RESULT SUMMARY

Negative: No clinically significant variant(s) detected

Gene(s) analyzed: *ALDOB* and *SLC12A3*

Recommendations:

Consideration of residual risk by ethnicity after a negative carrier screen is recommended, especially in the case of a positive family history for a specific disorder.

Interpretation:

Screening for the presence of pathogenic variants in the *ALDOB* and *SLC12A3* genes, which are associated with hereditary fructose intolerance and Gitelman syndrome, respectively, was performed by next generation sequencing and possibly genotyping for select variants on DNA extracted from this patient's sample. No clinically significant variants were detected during this analysis.

Please note that negative results reduce but do not eliminate the possibility that this individual is a carrier for the disorder(s) tested. Please see table of residual risks for specific detection rates and residual risk by ethnicity. With individuals of mixed ethnicity, it is recommended to use the highest residual risk estimate. Only variants determined to be pathogenic or likely pathogenic are reported in this carrier screening test.

Comments:

This carrier screening test masks likely benign variants and variants of uncertain significance (VUS) if there are any. Only known pathogenic variants or likely pathogenic variants which are expected to result in deleterious effects on protein function are reported. If reporting of likely benign variants and VUS is desired in this patient, please contact the laboratory (tel. 212-241-2537) to request an amended report.

Please note these tests were developed and their performance characteristics were determined by Mount Sinai Genomics, Inc. They have not been cleared or approved by the FDA. These analyses generally provide highly accurate information regarding the patient's carrier or affected status. Despite this high level of accuracy, it should be kept in mind that there are many potential sources of diagnostic error, including misidentification of samples, polymorphisms, or other rare genetic variants that interfere with analysis. Families should understand that rare diagnostic errors may occur for these reasons.

Patient: Donor 4991

DOB: [REDACTED]

Lab #: [REDACTED]

Table of Residual Risks by Ethnicity

Please note: This table displays residual risks after a negative result for each of the genes and corresponding disorders. **If a patient is reported to be a carrier of a disease, their residual risk is 1 and this table does not apply for that disease.**

Disease (Inheritance)	Gene	Ethnicity	Carrier Frequency	Detection Rate	Residual Risk	Analytical Detection Rate
Gitelman Syndrome (AR) NM_000339.2	SLC12A3	Worldwide	1 in 100	51%	1 in 203	>95%
Hereditary Fructose Intolerance (AR) NM_000035.3	ALDOB	Caucasian	1 in 80	95%	1 in 1581	>95%
		African	1 in 406	>95%	1 in 8101	>95%
		Hispanic	1 in 275	94%	1 in 4568	>95%
		Worldwide	1 in 121	87%	1 in 924	>95%

AR: Autosomal Recessive

This case has been reviewed and electronically signed by Anastasia Larmore, PhD, Assistant Director

Laboratory Medical Consultant: George A. Diaz, M.D., Ph.D.

Patient: Donor 4991	DOB: [REDACTED]	Lab #: [REDACTED]
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Test Methods and Comments

Genomic DNA isolated from this patient was analyzed by one or more of the following methodologies, as applicable:

Fragile X CGG Repeat Analysis

PCR amplification using Asuragen, Inc. AmpliX[®] *FMR1* PCR reagents followed by capillary electrophoresis for allele sizing was performed. Samples positive for *FMR1* CGG repeats in the premutation and full mutation size range are further analyzed by Southern blot analysis to assess the size and methylation status of the *FMR1* CGG repeat.

Genotyping

Multiplex PCR amplification and allele specific primer extension analyses using the MassARRAY[®] System or Luminex[®] xMAP[®] technology were used to identify variants that are complex in nature or are present in low copy repeat regions and are, therefore, not amenable to Next Generation Sequencing technologies. Rare sequence variants may interfere with assay performance.

Multiplex Ligation-Dependent Probe Amplification (MLPA)

MLPA[®] probe sets and reagents, MRC-Holland, were used for the analysis of copy number of specific targets versus known control samples. Each target region was assayed with two adjacent oligonucleotide probes which following hybridization were ligated and used as template for subsequent rounds of amplification. Each complete probe within the assay has a unique length and amplicons are separated and identified by capillary electrophoresis. False positive or negative results may also occur due to rare sequence variants in target regions detected by MLPA probes. Analytical sensitivity and specificity of the MLPA method are both 99%.

For Alpha Thalassemia, the copy numbers of the *HBA1* and *HBA2* genes were analyzed. Alpha-globin gene deletions, triplications, and the Constant Spring (CS) mutation are assessed. However, it does not detect all known alpha-thalassemia mutations such as point mutations. In addition, carriers of alpha-thalassemia with three or more *HBA* copies on one chromosome, and one or no copies on the other chromosome, will not be detected. This test detects most alpha-globin gene deletions, triplications, and the Constant Spring (CS) mutation using Multiplex Ligation-Dependent Probe Amplification (MLPA). It is expected to detect approximately 90% of all alpha-thalassemia mutations, varying by ethnicity. Therefore, this result reduces, but does not eliminate, the chance that this patient is a carrier of alpha-thalassemia. With the exception of triplications, other benign alpha-globin gene polymorphisms will not be reported.

For Duchenne Muscular Dystrophy, the copy numbers of all *DMD* exons were analyzed. Please note that single-exon deletions and duplications will not be reported unless they are confirmed by NGS data (for example, if breakpoints occurring in an exon can be visualized).

For Spinal Muscular Atrophy (SMA), the copy numbers of the *SMN1* and *SMN2* genes were analyzed. The individual dosage of exons 7 and 8 as well as the combined dosage of exons 1, 4, 6 and 8 of *SMN1* and *SMN2* were assessed. Copy number gains and losses can be detected with this assay.

Depending on ethnicity 6 - 29 % of carriers will not be identified by dosage sensitive methods as this testing cannot detect individuals with two copies (duplication) of the *SMN1* gene on one chromosome and loss of *SMN1* (deletion) on the other chromosome (silent 2+0 carrier) or individuals that carry an intragenic mutation in *SMN1*. Please also note that 2% of individuals with SMA have an *SMN1* mutation that occurred *de novo*. Typically in these cases, only one parent is an SMA carrier.

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The presence of the g.27134T>G variant allele in an individual with Ashkenazi Jewish or Asian ancestry is indicative of a duplication of *SMN1*. When present in an Ashkenazi Jewish or Asian individual with two copies of *SMN1*, g.27134T>G is likely indicative of a silent (2+0) carrier. In individuals with two copies of *SMN1* with African American, Hispanic or Caucasian ancestry, the presence or absence of g.27134T>G significantly increases or decreases, respectively, the likelihood of being a silent 2+0 silent carrier.

Next Generation Sequencing (NGS)

NGS was performed on a panel of genes for the purpose of identifying pathogenic or likely pathogenic variants.

Agilent SureSelect™QXT technology was used with custom capture library to target the guaranteed list of mutations and exonic regions of the relevant genes. These targeted regions were sequenced using the Illumina HiSeq2500 system with 100 bp paired-end reads. The DNA sequences were mapped to and analyzed in comparison with the published human genome build UCSC hg19 reference sequence. The targeted coding exons and splice junctions of the known protein-coding RefSeq genes were assessed for the average depth of coverage and data quality threshold values. This technology may not detect all small insertion/deletions and is not diagnostic for large duplications/deletions, repeat expansions, and structural genomic variation. This test will detect variants within the exons and the intron-exon boundaries of the target regions. Variants outside these regions will either not be detected or are not guaranteed to be detected. These regions include, but are not limited to, UTRs, promoters, and deep intronic areas or regions that fall within low copy repeat segments. In addition, a mutation(s) in a gene not included on the panel could be present in this patient. All potentially pathogenic variants may be confirmed by either a specific genotyping assay or Sanger sequencing, if indicated. Any benign variants, likely benign variants or variants of uncertain significance identified during this analysis were not reported.

Sanger Sequencing

Sanger sequencing, as indicated, was performed in both directions using BigDye Terminator chemistry with the ABI 3730 DNA analyzer with target specific amplicons. It also may be used to supplement specific guaranteed target regions that fail NGS sequencing due to poor quality or low depth of coverage <20 reads or as a confirmatory method for NGS positive results. False negative results may occur if rare variants interfere with amplification or annealing.

Tay-Sachs Disease (TSD) Enzyme Analysis

Hexosaminidase activity and Hex A% activity were measured by a standard heat-inactivation, fluorometric method using artificial 4-MU-β-N-acetyl glucosaminide (4-MUG) substrate. This assay is highly sensitive and accurate in detecting Tay-Sachs carriers and individuals affected with TSD. It is estimated that less than 0.5% of Tay-Sachs carriers have non-carrier levels of percent Hex A activity, and therefore may not be identified by this assay. In addition, this assay may detect individuals that are carriers of or are affected with Sandhoff Disease. False positive results, such as pseudodeficiency alleles, may occur if benign variants interfere with the enzymatic assay. False negative results may occur if both *HEXA* and *HEXB* pathogenic variants are present in the same individual.

This test was developed, and its performance characteristics determined by Sema4 Opco, Inc. It has not been cleared or approved by the US Food and Drug Administration. FDA does not require this test to go through premarket FDA review. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments (CLIA) as qualified to perform high complexity clinical laboratory testing. These analyses generally provide highly accurate information regarding the patient's carrier or affected status. Despite this high level of accuracy, it should be kept in mind that there are many potential sources of diagnostic error, including misidentification of samples, polymorphisms, or other rare genetic variants that interfere with analysis. Families should understand that rare diagnostic errors may occur for these reasons.

Patient: Donor 4991	DOB: [REDACTED]	Lab #: [REDACTED]
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SELECTED REFERENCES

Carrier Screening

Grody W et al. ACMG position statement on prenatal/preconception expanded carrier screening. *Genet Med.* 2013 15:482-3.

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Alpha-thalassemia:

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Chen L et al. An information-rich CGG repeat primed PCR that detects the full range of Fragile X expanded alleles and minimizes the need for Southern blot analysis. *J Mol Diag* 2010 12:589-600.

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Hendrickson BC et al. Differences in SMN1 allele frequencies among ethnic groups within North America. *J Med Genet.* 2009 46:641-4.

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Ashkenazi Jewish Disorders:

Scott SA et al. Experience with carrier screening and prenatal diagnosis for sixteen Ashkenazi Jewish Genetic Diseases. *Hum. Mutat.* 2010 31:1-11.

Duchenne Muscular Dystrophy:

Aartsma-Rus A et al. Entries in the Leiden Duchenne muscular dystrophy mutation database: an overview of mutation types and paradoxical cases that confirm the reading-frame rule. *Muscle Nerve.* 2006b 34:135-44.

Flanigan KM et al. Mutational spectrum of DMD mutations in dystrophinopathy patients: application of modern diagnostic techniques to a large cohort. *Hum Mutat.* 2009 30:1657-66.

Beta Globin-related Disorders:

Cao A et al. Beta-Thalassemia. GeneReviews (<http://www.ncbi.nlm.nih.gov/books/NBK1426/>)

Modell B et al. Epidemiology of haemoglobin disorders in Europe: an overview. *Scand J Clin Lab Invest.* 2007 67:39-69.

For further reading:

Orphanet: <http://www.orpha.net/consor/cgi-bin/index.php>

GeneReviews: <http://www.ncbi.nlm.nih.gov/books/NBK1116/>

For Disease Specific Standards and Guidelines:

<https://www.acmg.net/>

Additional disease-specific references available upon request.